205*

Microbiology results from a phase 2 clinical study of aztreonam lysinate for inhalation (AI): a new inhaled antibiotic to treat CF patients with *Pseudomonas aeruginosa (PA)*

J.L. Burns,[1,2] J. Stapp[2], D. Lofland[3], AI Phase II Study Group
[1]*University of Washington,* [2]*Therapeutic Development Network Core Microbiology Laboratory and* [3]*Corus Pharma Inc., Seattle, WA, USA*

Chronic *P. aeruginosa* airway infection is a major cause of morbidity and mortality in CF. Aerosol antibiotic delivery to the lungs is an attractive alternative to systemic administration. Tobramycin Inhalation Solution is approved for use in CF patients, but has been associated with decreased tobramycin susceptibility, based on minimal inhibitory concentrations (MIC). AI represents a new therapy specifically formulated for aerosol administration via an eFlow® Electronic Nebulizer. In a randomized, double-blind trial, CF patients (FEV_1 >40% predicted) received AI 75 mg (n=37), AI 225 mg (n=37), or placebo (n=31) BID for 14 days. Sputum samples were collected on days 0, 7, 14 and 28 for quantitative culture and susceptibility testing. At days 7 and 14, CFU density decreased >1.5 $\log_{10}$ for AI 75 mg and >2.1 $\log_{10}$ for AI 225 mg (P <0.001). Baseline *P. aeruginosa* MIC_{50} and MIC_{90} for AI were ≤1 and 16 µg/mL for placebo, 2 and 64 µg/mL for AI 75-mg, and ≤1 and 128 µg/mL for AI 225-mg groups, respectively. At day 14, there was no increase in MIC_{50} or MIC_{90} with placebo or AI 75-mg, and only a slight increase in MIC_{50} with AI 225 mg. At day 28, MIC_{50} returned to baseline in placebo and AI 225-mg groups, and a 1-dilution increase was seen in AI 75-mg. MIC_{90} increased 1-dilution from baseline for placebo and AI 75 mg, but was unchanged for AI 225-mg. AI appeared to effectively reduce bacterial load and increased resistance was not apparent with 14 days treatment with ≤225 mg AI BID. AI could provide an important option in the management of PA in CF patients. Further study of the safety and efficacy of AI is ongoing.

206

Evaluation of the Etest for the assessment of synergy of antibiotic combinations against multi-resistant *Pseudomonas aeruginosa* isolates from Cystic Fibrosis patients

B. Balke[1], M. Hogardt[2], S. Schmoldt[2], L. Hoy[3], H. Weissbrodt[1], S. Häussler[4]
[1]*Institut für Medizinische Mikrobiologie und Krankenhaushygiene, Hanover, Germany,* [2]*Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Munic, Germany,* [3]*Institut für Biometrie, Hanover, Germany,* [4]*Gesellschaft für Biotechnologische Forschung (GBF), Braunschweig, Germany*

The determination of synergistic effects of antimicrobial combinations can lead to improved therapeutic options in the antibiotic treatment of cystic fibrosis (CF) patients, who are chronically infected with multi-resistant *Pseudomonas aeruginosa* isolates. In this study we evaluated the performance of the Ellipsometer test (Etest) in the assessment of synergy in comparison to the standard agar dilution checkerboard susceptibility test and determined the activity of two antimicrobial combinations against 163 multi-resistant *P. aeruginosa* CF isolates. The agreement between the checkerboard and the Etest susceptibility test method was excellent (>90%) for both, non-mucoid and mucoid CF strains. The highest rate of synergy was observed for the antibiotic combination of ceftazidime and tobramycin (28.8% of the CF strains) as opposed to the antibiotic combination of meropenem and tobramycin (19.0%). However, the probability of synergy for the second antibiotic combination increased significantly when for the first antibiotic combination synergy had already been demonstrated (Fischer's exact test p=0.049). We conclude that the Etest is a valuable and practical method for routine microbiological diagnostics improving the antibiotic options in the treatment of CF patients chronically infected with *P. aeruginosa*.

207

In Vitro Study of Colistin and Tobramycin Effectiveness against Gram(-) Bacteria obtained from Cystic Fibrosis Patients

A. Bisiklis[1], M. Fotoulaki[2], P. Tsioutsias[1], St. Alexiou-Daniel[1], S. Nousia-Arvanitakis[2]
[1]*Department of Microbiology, AHEPA University Hospital, School of Medicine, Thessaloniki, Greece;* [2]*Fourth Pediatric Department, Aristotle University of Thessaloniki, Greece*

OBJECTIVE: The purpose of the present study was to determine tobramycin and colistin effectiveness against Pseudomonas aeruginosa and Achromobacter xylosoxidanas strains in vitro.
MATERIALS AND METHODS: Forty four Pseudomonas aeruginosa strains and 5 Achromobacter xylosoxidas strains isolated from sputum cultures obtained from patients with cystic fibrosis were studied. The identification was performed by API 20NE. The determination of effectiveness of colistin and tobramycin was performed by E-test (AB BIODISK). Strains were considered resistant to tobramycin and colistin when the minimum inhibition concentrates (MICs) were ≥16µg/ml and ≥32µg/ml, respectively.
RESULTS: Pseudomonas aeruginosa strains revealed 75% sensitivity to tobramycin and 100% to colistin, respectively. One out of five Achromobacter xylosoxidans strains was sensitive to tobramycin and colistin, another strain was resistant to both antibiotics. The remaining three strains were resistant to tobramycin and sensitive to colistin.
CONCLUSIONS: a) All Pseudomonas aeruginosa strains appeared sensitive to colistin and 75% were sensitive to tobramycin.b) Achromobacter xylosoxidans had decreased susceptibility to colistin. This is an interesting point as no patient in the present study was under treatment with colistin.

208

The results of antibiotic synergy testing of resistant isolates of *Pseudomonas aeruginosa* correlate poorly with clinical outcome in acute exacerbation of cystic fibrosis

J. Foweraker[1], C. Laughton[1], D. Brown[3], E. Gunn[2], D. Bilton[2]
[1]*Department of Microbiology, Papworth Hospital, Cambridge, UK,* [2]*Adult CF Centre, Papworth Hospital, Cambridge, UK,* [3]*Department of Microbiology, Addenbrookes Hospital, Cambridge, UK*

Patients with acute exacerbation of CF may improve on antibiotics yet have resistant *Pseudomonas aeruginosa* (*PA*) in sputum. This may be due to a synergistic effect of the antibiotic combinations used to treat *PA*. Our aim was to test multiple isolates of *PA* from sputum taken before starting treatment, select those resistant to both antibiotics and look at the predictive value of synergy testing on treatment outcome.
Adults with CF, colonised with *PA* were treated for an exacerbation with two antibiotics for 14 days. Clinical response by days 7 and 14 was measured by change in FEV_1 and daily sputum weight. In 9 exacerbations in 7 patients, one or more isolates of *PA* resistant to both prescribed antibiotics was found. The combinations used were tobramycin plus ceftazidime, aztreonam, meropenem or piperacillin/tazobactam. Antibiotic interactions were tested in vitro using conventional synergy methods of checkerboard and time kill curves and the recently described Multiple Combination Bactericidal Test.
The results of synergy testing of the same isolate were different by the various methods.6 patients had more than one resistant isolate of *PA* in the same sputum with examples showing both indifference and synergy and a wide range of chequerboard synergy indices. No test reliably predicted the clinical outcome of treatment. This suggests that either the resistant isolates of *PA* were not the cause of the symptoms or that we lack a method of *in vitro* synergy testing that predicts the clinical effect of antibiotic combination in the treatment of infection with *PA* in CF.